Fungal succession on aspen poplar leaf litter

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Fungi from the litter, fermentation, and humus (LFH) layers of a mature aspen tree stand were qualitatively assessed using the soil-washing method and quantitatively assessed using the agar-film technique. Fungi were isolated and identified at two sampling times, June and October. From the isolation data obtained, it was concluded that the pattern of fungal succession in aspen litter followed the general succession schemes proposed by Garrett (1963) and Hudson (1968). The parasites on living aspen leaves included a sterile dark organism and *Pleurophomella spermatiospora*, while the common primary saprophytes, thought to be existing on sugars and simple carbon compounds in the leaf, included *Penicillium janthinellum, Cladosporium* spp., and *Aureobasidium pullulans*. The secondary saprophytes, such as the cellulose and lignin decomposers and associated fungi, were dominated by *Penicillium syriacum, Trichoderma* spp., *Mucor* spp., *Volutella ciliata, Cylindrocarpon* spp., and *Phoma* spp.

The quantitative study revealed that the fungal length and biomass per gram dry weight of litter in each layer studied were much higher in October than in June. The amount of fungal mycelium in the humus in each of the two sampling times was not significantly different. The difference in standing crop of mycelium from June to October in the aspen LFH horizon was estimated to be at least 58 g wet mycelium per square metre of dry litter.

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Les auteurs ont estimé qualitativement, à l'aide de la méthode délavage du sol, et quantitativement par la méthode du film d'agar, la population des champignons dans la litière ainsi que dans les horizons de fermentation et d'humification (LFH) d'un peuplement mature de *Populus tremuloides*. L'isolement et l'identification des champignons ont été effectués à deux périodes d'échantillonnage, soit en juin et en octobre. Les résultats des isolements permettent de conclure que le patron de la succession fongique dans la litière de *P. tremuloides* suit le schème général de succession proposé par Garrett (1963) et Hudson (1968). Les parasites sur les feuilles vivantes de *P. tremuloides* comprennent entre autres un mycélium stérile foncé et *Pleurophomella spermatiospora*, alors que les saprophytes primaires communs, qu'on croit généralement tributaires des sucres et des produits carbonés simples de la feuille, sont représentés par *Penicillium janthinellum, Cladosporium* spp. et *Aureobasidium pullulans*. Les saprophytes secondaires tels que les décomposeurs de la cellulose et de la lignine et leurs champignons associés sont dominés par *Penicillium syriacum, Trichoderma* spp., *Mucor* spp., *Volutella ciliata, Cylindrocarpon* spp. et *Phoma* spp.

L'étude quantitative a montré que la longueur du mycélium et la biomasse fongique par gramme de poids sec de litière dans chaque couche étudiée sont beaucoup plus élevées en octobre qu'en juin. La quantité de mycélium fongique dans l'humus ne présente pas de différence significative entre les deux périodes d'échantillonnage. La différence dans la quantité de mycélium entre juin et octobre dans l'horizon LFH a été estimée à au moins 58 g de mycélium frais par mètre carré de litière sèche. [Traduit par le journal]

Introduction

Since 1950, when Chesters described the succession of ascomycetes on decaying deciduous logs and branches, a number of studies have attempted to describe the fungal successional patterns found on various types of decomposing plant debris on the soil surface. Hudson (1968) published a detailed review of all the major work investigating these fungal successional patterns.

Fungal succession studies on deciduous tree leaf litter were initiated by Saito (1956) in his investigation of the decomposition of beech leaves. Hering (1965) using damp-chamber incubation (Webster 1956, 1957), direct observation, and isolation from washed fragments (Harley and Waid 1955) after surface-sterilization carried out an intensive survey of the fungi on leaves of known age from oak, hazel, birch, and ash. A similar study was performed by Hogg and Hudson (1966) on the succession of fungi on

living and dead leaves of beech. In addition to using the damp-chamber and cultural-isolation techniques, Hogg and Hudson (1966) also suspended leaves over nutrient agar in an attempt to isolate ascospore- or ballistospore-producing fungi. Watson *et al.* (1974) have recently published a fungal succession study on loblolly pine and upland hardwood (mostly blackjack oak) foliage and litter. They compared the fungi directly observed in damp chambers and isolated from washed and surface-sterilized plant fragments of pine and oak foliage and litter. Although some of these successional studies have been extensive and have frequently paid considerable attention to the techniques of isolation of fungi, there has been little emphasis placed on statistical analysis of fungal isolation data to obtain a more accurate picture of the frequencies of occurrence and statistical significance of the fungi involved in the various stages in a particular succession.

Other recent publications concerned with microfungi isolated from deciduous woodlands include a study by Morrall (1974) on the fungi associated with aspen poplar stands and one by Wicklow *et al.* (1974) on fungi associated with alder, conifer (Douglas fir, western hemlock, and sitka spruce), and mixed alder–conifer stands. However, neither of these studies was aimed at assessing successional patterns on deciduous tree leaf litter but, rather, concentrated on correlating environmental factors or vegetation type with the distribution of fungal isolates.

Quantitative studies on fungi decomposing deciduous tree litter have been much less frequent than qualitative studies. However, because of the importance of decomposer biomass data for estimates of energy flow and nutrient cycling, this deficiency is being rectified in several integrated ecosystem studies. In the restricted number of already published studies, various methods for direct microscopic measurement of hyphae on, or released from, leaf litter samples have been used (Minderman and Daniels, 1967; Nagel-de Boois and Jansen 1971). Recently the adenosine 5′-triphosphate (ATP) assay technique has been used in an attempt to estimate bacterial and fungal biomass in litter and soil (Ausmus and Witkamp 1974).

Because of the sparse quantitative data from the litter, fermentation, and humus (LFH) horizon in deciduous woodlands, one of the aims of this study was to measure the quantitity of fungal mycelium in the layers of this horizon in an aspen poplar (*Populus tremuloides* Michx.) woodland. Associated with this quantitative study was an extensive survey of the fungi isolated from the live aspen poplar leaves through the litter and fermentation layers to the humus layer. Both the quantitative and qualitative data collected in this study were subjected to a detailed statistical analysis and a fungal successional pattern during the decomposition of aspen poplar leaf litter is proposed.

Description of Study Area

The sampling area is located in the Kananaskis Valley in the front range of the Rocky Mountains (51°2′ N, 115°2′ W) in Alberta, Canada, at an altitude of 1430 m.

The climate of the area is continental with a mean annual temperature of 4°C and a mean annual precipitation of 64 cm. The winter climate (October to March) is characterized by alternating cold, dry periods and warm, dry, windy chinook periods, while the summers are relatively short and dry. The soil is frozen from mid-November to mid-April and is covered with snow from mid-December to mid-April. Soil moisture is highest during snowmelt and during periods of spring and early summer rain.

The dominant tree species in the area is *Populus tremuloides* Michx., which is associated with *Populus balsamifera* L. and *Pinus contorta* Dougl. The shrub understory includes *Rosa acicularis* Lindl. and *Rosa woodsii* Lindl., while the herbaceous understory includes *Heracleum lanatum* Michx., *Epilobium angustifolium* L., *Geranium richardsonii* Fisch. and Trautv., *Castilleja miniata* Dougl., *Viola*, *Smilacina*, *Galium*, and *Delphinium*, plus a mixture of grasses.

The soil profile is well developed and is classified in the orthic gray luvisol subgroup of soils (Karkanis 1972). The organic horizon varies in depth from 5 to 9 cm and can easily be separated into four distinct layers in spring and five layers in the fall as follows.

(*i*) Litter (L_1 and L_2), 2–3 cm deep; pH 7.0–7.5; consisting of whole brown leaves and other herbaceous litter that have been only slightly decomposed. In the fall the litter layer can be divided into the L_1, which consists of the leaves that have just fallen from the trees, and the L_2, which consists of the leaves that fell the previous year.

(*ii*) Fermentation 1 (F_1), 1–2 cm deep; pH 6.5–

7.0; consisting of dark brown to black leaves in a more advanced state of decomposition. Fungal mycelia in the form of mycelial strands are quite common in this matted layer.

(*iii*) Fermentation 2 (F_2), 1–2 cm deep; pH 6.0–7.0; consisting of fragmented black leaves of which the botanical origin is still discernible. Fungal mycelia, mycelial strands, and faecal pellets of soil fauna are abundant in this layer.

(*iv*) Humus (H), 1–2 cm deep; pH 6.0–6.5; consisting of brown to black, amorphous, sticky, organic matter decomposed to such a degree that the botanical origin of the organic material is not discernible. Large numbers of rootlets make the humus fibrous in nature.

The percentage organic matter as measured by loss on ignition at 600°C ranged from 91% in the L to 79% in the H.

The mineral soil profile is made up of Ah, Ae, Bt, and Ck horizons (Karkanis 1972).

Methods

Sampling

Description of the Sampling Unit

A sampling area, consisting of a 30 m × 30 m square plot, was staked out in a mature aspen stand. Within this sampling area, five 6 m × 6 m subplots were measured, one subplot was placed in each corner of the sampling area and one was placed in the center. Each of the 6 m × 6 m subplots was then subdivided into nine 2 m × 2 m units and the units were numbered 1 to 9.

Selection of Samples for Qualitative and Quantitative Analysis of the Fungal Populations

At each of the sampling times (June and October) one of the 4-m^2 units in each of the five subplots was randomly selected. From each of the five selected units a sample of material was taken from each of the layers in the organic horizon. In June the following sampling procedure was followed: 500–600 leaves per sample were collected from the L layer; 200–300 leaves per sample were collected from the F_1 layer; and 500–700 g material was collected from each of the F_2 and H layers. As well as sampling from the organic layer, five samples of live leaves were taken (200–300 leaves per sample).

In the October sampling period similar sampling procedures were used. However, at this time the L layer was divisible into L_1 and L_2 (as described previously); therefore 500–600 leaves were taken from the L_1 and L_2 in each of the five sample units. Samples from the F_1, F_2, and H layers were taken as in the June sampling time. An attempt was also made to collect leaves immediately after leaf fall, but before they had made contact with the litter surface. Therefore, just before leaf fall, large nylon nets were suspended from the trees in the vicinity of each of the five subplots. The nets were placed in such a way that they would not disturb the accumulation of the L_1 in the units from which the other samples were to be taken. From the leaves collected in these nets, five samples, which consisted of 500–600 leaves each, were removed.

Immediately after sampling in both June and October, the samples were transported to the laboratory in polyethylene bags, stored at 2 to 3°C, and qualitative and quantitative analysis of fungal populations made as quickly as possible thereafter.

Analysis of the Fungal Populations

Qualitative Studies

Because it has been demonstrated that washing rids roots, plant debris, and soil of easily removable fungal propagules such as fungal spores (Harley and Waid 1955; Williams *et al.* 1965; Parkinson and Balasooriya 1967), it was decided that the isolations of fungi from the L, F, and H layers would be effected using the washing technique described by Bissett and Widden (1972).

(a) Live, Net-caught, L_1, and L_2 Leaf Samples

The live and L leaves sampled in the spring and the net-caught, L_1, and L_2 leaves sampled in the fall were macerated in a Waring blendor for 3 min and the macerate was sieved through a 2-mm screen.

Before the use of maceration in this study, a preliminary investigation was performed to examine whether maceration of leaves released toxic compounds that could inhibit the growth of some fungi developing from the washed leaf particles; such inhibition has been recorded when using macerates of various types of plant material. However, no significant difference was observed between the fungi isolated from macerated L leaf particles that had been subjected to 50 1-min washings and the fungi isolated from particles cut with scissors from 8-mm leaf discs that had also been given 50 1-min washings. Since maceration of leaves in the Waring blendor resulted in a more homogeneous mixture of leaf particles and also in reduction of the risk of contamination, it was adopted as the procedure for obtaining small leaf fragments for subsequent plating onto the isolating medium.

After maceration and sieving, 0.5-g samples of those leaf particles that had an area of 2 mm^2 or less were weighed out and given 15 1-min washings with sterile water. The number of washings used was shown in a preliminary washing efficiency study (i.e., that used by Harley and Waid 1955) to be optimal for the removal of detachable fungal propagules (e.g., spores) from the leaf particles. After washing, excess water was removed from the fragments using sterile filter paper, and 100 leaf particles from each sample were placed on Czapek-Dox agar (pH 4.5); two leaf particles were inoculated onto each plate. As a result, 500 particles from each organic layer under study were plated at each sampling time. The plates were incubated at room temperature for 2 to 6 weeks, and identification of the fungi developing from each plated leaf particle was attempted.

(b) F_1, F_2, and H Layer Samples

The F_1 leaf samples were macerated and sieved as described previously while the F_2 and H layer samples were only sieved through a 2-mm screen. Particles of the F_1, F_2, and H that had an area of 2 mm^2 or less were then weighed into 1-g samples and given 20 1-min washings with sterile water (a preliminary washing efficiency study indicated 20 washings were necessary to remove most fungal spores from F_1, F_2, and H samples). Washed particles were plated (500 particles from each of the F_1, F_2, and H layers at each of the two sampling times) and

were incubated in the same manner as that described for the live and L leaves.

The total number of particles plated for fungal isolations at each of the two sampling times was 2500 in June and 3000 in October. After recording the fungi developing from each plated particle, the percentage frequency of occurrence ((number of particles on which fungus occurred per total number of particles plated) × 100) was calculated for each fungal species in each of the organic layers under study in each of the five replicate units sampled at each sampling time. Means were then calculated for the five figures of occurrence for each fungus isolated in each of the organic layers and the occurrence data for the fungal isolates were analyzed for their statistical significance of occurrence in each layer at each sampling time in an attempt to obtain a successional scheme for fungi decomposing aspen leaf litter in the spring and in the fall.

Quantitative Assessment of Fungal Mycelium

(a) Live, Net-caught, L_1, L_2, F_1, and F_2 Leaf Samples

The methods of quantitative assessment of fungi in mineral soil samples have been reviewed by Nicholas and Parkinson (1967), and it has been concluded that the Jones and Mollison agar-film technique (1948) is the best method yet available. The applicability of this method for assessments of amounts of fungal mycelium in decomposing litter has been a matter of dispute. Therefore, at the outset of this investigation an attempt was made to assess the value of various direct observation methods for the quantitative study of fungi in the litter layers.

Leaf-clearing techniques developed by Janes (1962), Hering and Nicholson (1964), and Minderman and Daniels (1967) were applied to whole aspen leaves. The methods of Janes (1962) and Hering and Nicholson (1964) were found to be unsuitable for aspen litter because of excessive damage caused in some cases to the leaf tissue as a result of the chemical treatment involved. However, the leaf-clearing procedure developed by Minderman and Daniels (1967) in which oak leaf discs were fixed in 4% formaldehyde for 24 h, bleached in 6% hydrogen peroxide, and washed and stained in chlorazol-black proved to be applicable to aspen leaves with the variation that aspen leaf discs required a 10% concentration of hydrogen peroxide to effect successful bleaching. However, comparison of data on mycelial lengths in aspen L layer litter samples observed by use of the Minderman and Daniels (1967) leaf-clearing technique with similar data obtained from samples using the unmodified Jones and Mollison (1948) agar film showed that the mycelial length ± standard error (SE) on cleared leaf discs totalled 485 ± 56 m/g dry litter, while the mycelial length ± SE on agar films amounted to 796 ± 118. Thus, the use of the conventional preparation of agar films, normally applied to mineral soil, gave a higher estimate of mycelial lengths in leaf litter. However, it was felt that the use of this method, which involved grinding leaves in a mortar and pestle, only allowed assessment of lengths of mycelium present on leaf surfaces. Mycelium within the leaves remained attached to the leaf tissue and was consequently not measured, resulting in an underestimate of the total mycelial length in the L layer. In an effort to release the mycelium present within the leaves into the supernatant, the Waring blendor was used to grind the leaves. When agar films were made after litter samples had been macerated in a Waring blendor at 10 000 rpm for 3 min (a speed and time shown in test experiments to be optimal for release of mycelium from this type of organic material), the measured mycelial length (metres per gram dry aspen litter) ± SE was 2975 ± 205 as compared with 796 ± 118 m/g dry litter obtained by grinding leaves in a mortar and pestle. Since the vigorous action of the Waring blendor proved to be a better means for releasing mycelium from whole leaves, this method was used for suspending mycelium in water before making agar films. The modified Jones and Mollison (1948) slide treatment of live, net-caught, L, L_1, L_2, F_1, and F_2 layer leaf samples was as follows.

Two-gram leaf samples (five replicates from each layer under study at each of the two sampling times) were macerated in 100 ml of sterile distilled water in a Waring blendor (10 000 rpm) for 3 min. Fifty millilitres of the litter–water macerate was then decanted into a flask, and 50 ml of molten 3% water agar was added to the macerate, which resulted in a 1/100 dilution. Agar films were made according to the technique described by Jones and Mollison (1948) and by incorporating modifications suggested by Thomas *et al.* (1965); however, films were left unstained. From each replicate leaf sample, 10 slides were prepared. One hundred randomly chosen fields were observed from each slide prepared from the live and net-caught leaf samples, while only 50 fields were observed from each slide prepared from the L_1, L_2, F_1, and F_2 leaf samples. In all cases observation of agar films was made using phase-contrast microscopy (using a drawing attachment). Mycelium, observed in each of the fields, was drawn, measured, and converted to metres per gram dry weight of leaf material. The average diameter of hyphae in each organic layer was determined and the volume of the measured mycelium calculated. Hyphal lengths could then be expressed as fungal biomass by multiplying the volume by the average specific gravity of mycelium obtained from figures quoted by Saito (1955). Since the dry weight of 1 m^2 of each organic layer had been calculated from a previous study, mycelial lengths were consequently converted to grams mycelium per square metre.

(b) H Layer Samples

One-gram humus samples (five replicates at each of the two sampling times) were analyzed quantitatively by applying the unmodified Jones and Mollison (1948) slide technique in which a 1/100 dilution of the humus material was used. The agar films were not stained but were treated in the same way as that described for the other organic layer samples. Five slides were made from each replicate sample, 50 fields were observed from each slide, and mycelial lengths were expressed in the same terms as described previously.

Statistical Analysis of the Qualitative and Quantitative Data

A two-way analysis of variance (ANOVA) without replication was applied to both the qualitative and quantitative data collected at each of the sampling times (June and October) to test for the significance of variation among the organic layers and among the five units sampled. The five spatial soil samples were then treated as statistical replicates, means of the mycelial lengths and

TABLE 1

Percentage frequency of occurrence of fungi isolated from the aspen poplar LFH horizon in June. (Figures represent means from five replicate samples from each layer)

Fungi isolated	Live leaves	Litter	F_1	F_2	H
Sterile dark LL1	23.0	0.0	0.0	0.0	0.0
Pleurophomella spermatiospora v. Höhn. Fragm. z. Myk	4.2	0.0	0.0	0.0	0.0
Aureobasidium pullulans (de Bary) Arnaud.	8.0	0.6	0.0	0.0	0.0
Beauveria bassiana (Bals.) Vuill.	0.2	6.2	0.0	0.0	0.0
Cladosporium spp. (*cladosporioides* (Fresen.) de Vries, *herbarum* (Pers.) Link ex S. F. Gray, *sphaerospermum* Penzig)	6.4	2.4	0.2	0.0	0.0
Basidiomycete spp.	0.0	2.2	0.0	0.0	0.0
Sterile dark forms	2.8	67.0	9.2	0.0	0.0
Phialophora fastigiata (Lagerb. & Melin) Conant	0.0	7.4	0.0	0.0	0.0
Paecilomyces farinosus (Dickson ex Fr.) Brown and Smith	0.0	12.2	3.8	11.2	17.2
Penicillium syriacum Baghdadi	0.0	0.8	48.4	34.6	12.2
Penicillium spp.	0.8	1.6	35.0	27.4	26.4
Phoma spp. (*cava* Schulzer, *exigua* Desm.)	2.4	32.4	12.6	37.8	30.8
Acremonium spp.	0.4	1.6	0.6	3.4	7.4
Trichoderma spp. (*hamatum* (Bon.) Bain., *harzianum* Rifai, *polysporum* (Link ex Pers.) Rifai, *viride* Pers. ex S.F. Gray)	0.0	0.8	49.4	65.2	75.0
Verticillium spp.	0.0	0.6	0.2	2.4	0.4
Discula spp.	0.0	4.0	14.0	1.6	0.0
Absidia spp.	0.0	0.0	11.8	19.8	9.8
Mucor spp.	0.0	0.0	5.4	7.2	1.2
Mortierella spp.	0.0	0.0	0.4	2.4	4.4
Cylindrocarpon spp. (*destructans* (Zins.) Scholten, *tenue* Bugn.)	0.0	0.0	0.2	8.8	19.8
Volutella ciliata (Alb. & Schw.) Fr.	0.0	0.0	0.6	6.0	16.0
Acremonium murorum (Corda) W. Gams	0.0	0.0	0.2	0.0	1.6
Dictyostelium sp.	0.0	0.0	0.0	1.6	0.4

the number of occurrences for each fungus isolated were computed, and the Student-Newman-Keuls (SNK) test was applied to test for the differences among these means. Before applying the ANOVA and SNK test, Bartlett's test and the F-max test were applied to the data to test for homogeneity of variances (one of the assumptions of the ANOVA to which the data must conform before the ANOVA and SNK test can be used). These tests indicated that variances obtained for the fungal occurrences and mycelial lengths were significantly heterogeneous, and therefore, to satisfy the assumptions of the analysis of variance, the data were transformed into another measurement scale. The data were tested with the following transformations: arcsine, square root, square root of $(x + 0.5)$, logarithm of $(x + 1)$, argsinh, and argsinh of $(x + 0.5)$. The means and variances of the fungal isolates and mycelial lengths were calculated for each transformation, and the variances were then tested for homogeneity by the Bartlett and F-max tests. The square-root transformation was most successful in rendering the sample variances, obtained from the spring and fall isolation data, homogeneous and independent of the sample means, while the quantitative data conformed to the assumptions of the analysis of variance when the original data were transformed into the argsinh scale.

Results and Discussion

Qualitative Data

The qualitative data obtained from isolating fungi from the Kananaskis aspen site in both the spring and fall are summarized in Tables 1 and 2. Only those organisms occurring with a frequency greater than 5% in any one sample have been listed. The number of occurrences in each layer for each fungus mentioned in Tables 1 and 2 is the mean of the number of occurrences in the five replicate samples. Figures 1 and 2 are

TABLE 2

Percentage frequency of occurrence of fungi isolated from the aspen poplar LFH horizon in October. (Figures represent means from five replicate samples from each layer)

Fungi isolated	Net-caught leaves	L_1	L_2	F_1	F_2	H
Penicillium janthinellum Biourge	74.2	0.0	0.0	0.0	0.0	0.0
Paecilomyces ochraceous Onions & Barron	4.6	0.0	0.0	0.0	0.0	0.0
Aureobasidium pullulans	5.8	4.8	0.0	0.0	0.0	0.0
Pleurophomella spermatiospora	0.2	6.0	0.0	0.0	0.0	0.0
Cladosporium spp. (*herbarum, cladosporioides, sphaerospermum*)	8.6	23.8	0.8	0.0	0.0	0.0
Alternaria spp.	0.6	0.2	2.8	0.2	0.0	0.0
QL2	0.0	2.4	10.6	3.2	0.0	0.0
Beauveria bassiana	0.0	7.0	1.6	0.8	0.0	0.0
Sclerotium sp.	0.0	0.0	2.4	0.4	0.0	0.0
Myrothecium inundatum Tode ex Fr.	0.0	0.2	7.8	4.0	0.8	0.0
Sterile hyaline forms	11.4	4.8	0.6	0.2	0.0	0.8
Phoma spp. (*cava, exigua*)	0.2	38.2	27.0	34.2	44.0	20.8
Trichoderma spp. (*hamatum, viride, polysporum*)	0.8	2.2	8.4	25.2	57.0	71.6
Acremonium murorum	0.4	0.0	0.0	0.6	3.0	1.8
Sterile dark forms	1.2	24.6	18.6	6.0	1.4	2.0
Penicillium spp.	14.0	13.8	7.0	1.0	3.8	29.8
Discula spp.	0.0	10.2	20.0	5.2	0.2	0.2
Mortierella spp.	0.0	21.8	6.6	2.2	2.6	25.6
Phialophora spp.	0.0	8.8	0.0	0.2	0.0	0.6
Cylindrocarpon spp. (*destructans, tenue*)	0.0	0.0	0.0	0.4	0.2	19.2
Mucor spp.	0.0	0.2	24.4	20.6	7.0	0.2
Paecilomyces spp.	0.0	1.0	4.0	0.6	0.2	0.6
Absidia spp.	0.0	0.4	1.6	15.2	20.6	1.4
Acremonium spp.	0.0	1.2	1.0	6.6	31.4	1.4
Penicillium syriacum	0.0	0.0	59.8	81.4	68.4	4.4
Paecilomyces farinosus	0.0	0.0	34.0	18.0	5.2	7.2
Verticillium spp.	0.0	0.0	2.6	3.4	1.8	1.4
Dictyostelium sp.	0.0	0.0	0.4	0.6	4.8	0.4
Volutella ciliata	0.0	0.0	0.6	0.2	10.6	16.4

histograms representing the fungal succession in the aspen LFH horizon. The histograms represent the mean frequency of occurrence for each fungus that had a frequency of occurrence greater than 5% in any one replicate and displayed a statistically significant ($P = 0.05$) difference between two or more of the layers sampled. Where the occurrence of a fungus significantly ($P = 0.05$) changed from one layer to the next has been represented by a solid line, while the broken line indicates no significant change in the occurrence of a particular isolate from one layer to the next. Most of the fungi that had a frequency of occurrence greater than 5% in one soil replicate did not display any statistically significant spatial variation (i.e., between subplots). *Volutella ciliata* in the June sample and *Trichoderma* spp., *Paecilomyces* spp., and *Acremonium* spp. in the October sample were the only isolates that varied significantly ($P = 0.05$) among the five subplots.

The fungi that were not sporulating when isolated were classified as sterile dark or sterile hyaline species and were given a code number. The sterile dark species listed as QL2 in Table 2 is a member of the Sphaeropsidales, which has also been isolated from Kananaskis pine litter (Widden and Parkinson 1973). Each isolated organism was, if possible, isolated into pure culture and stored.

The qualitative data tabulated and illustrated diagrammatically in Tables 1 and 2 and Figs. 1 and 2 indicate that the colonization of aspen leaves by fungi begins soon after the leaves

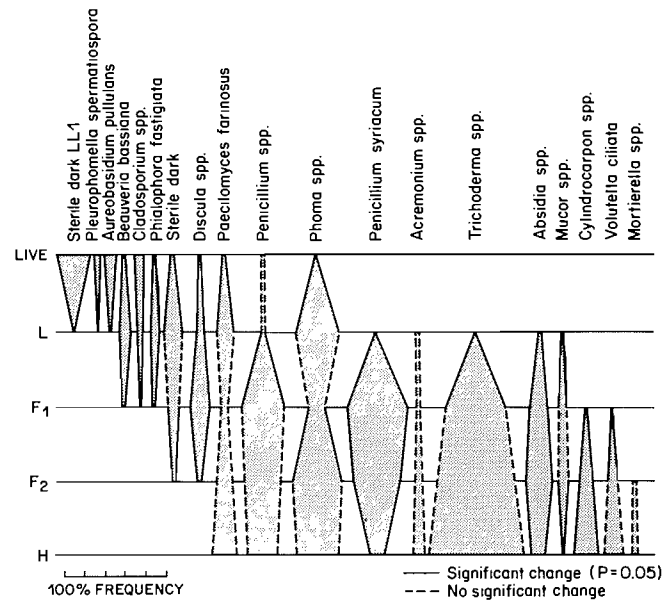

FIG. 1. Frequency and significance of fungi isolated from the live leaves to the humus layer in the aspen poplar site in June.

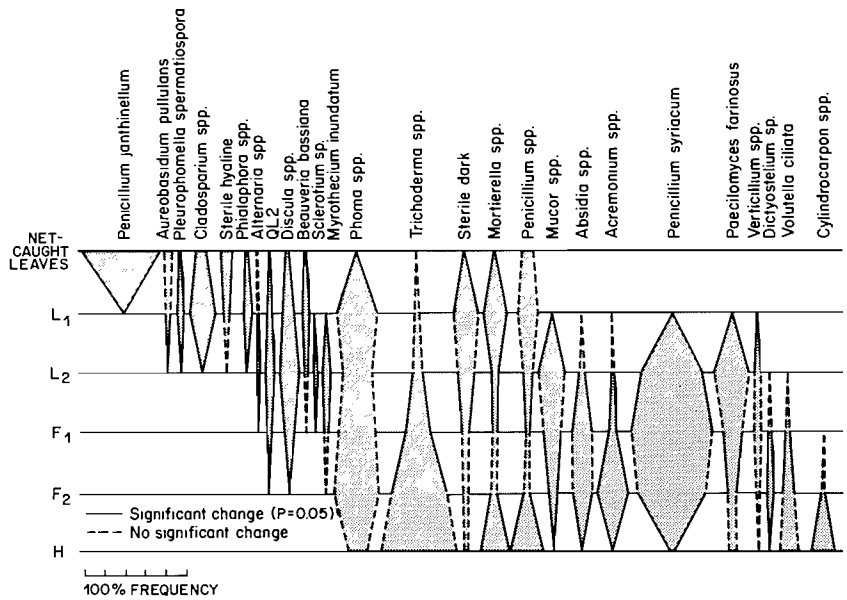

FIG. 2. Frequency and significance of fungi isolated from the net-caught leaves to the humus layer in the aspen poplar site in October.

emerge from the bud. The dominant fungi isolated from live leaves about 2 weeks after the leaves had emerged included a sterile dark fungus labelled LL1 (a dematiaceous organism which produces chains of chlamydospore-like structures), *Pleurophomella spermatiospora*, *Aureobasidium pullulans*, and *Cladosporium* spp. (*cladosporioides*, *herbarum*, *sphaerospermum*). *Cladosporium* and *Aureobasidium* have been recognized as primary colonizers that have been isolated from the live leaves of a number of deciduous tree species (e.g., live beech leaves

(Hogg and Hudson 1966); live oak, hazel, ash, and birch leaves (Hering 1965)). However, the occurrence of the sterile dark fungus (LL1) and of *P. spermatiospora* appears to be peculiar to living aspen leaves. In addition, in terms of the successional scheme for colonization of leaves by fungi presented by Hudson (1968), the sterile dark fungus (LL1) and *P. spermatiospora* may be considered to be host-specific or host-restricted parasites, while the *Cladosporium* spp. and *A. pullulans* represent common saprophytes existing on damaged leaf tissue or possibly on exudates from the live leaves.

After leaf fall but before colonization by the litter-inhabiting fungi (i.e., on net-caught leaves), *A. pullulans* and *Cladosporium* spp. occurred with about the same frequency as observed on the live leaves in the spring, the frequency of occurrence of *P. spermatiospora* decreased slightly, and sterile dark LL1 occurred very rarely. The most frequently recorded fungus on the net-caught leaves was *Penicillium janthinellum* (72% frequency of occurrence). The presence of this organism on net-caught leaves may have been due to a heavy snowfall, which at the time of leaf fall, could possibly have brought the organism in close contact with the leaf surfaces. This species of *Penicillium* plus the other *Penicillium* spp. isolated from net-caught leaves are probably members of the primary saprophytic fungi, whose existence depends upon their ability for rapid growth and utilization of easily available sugars and other carbon compounds in the absence of large competitor populations. A number of sterile hyaline fungi were also found colonizing aspen leaves before they reach the forest floor.

The fungi isolated from net-caught leaves are possibly stage 1*a* or stage 1 decomposers according to Garrett's (1963) simplified succession scheme. Live aspen leaves are colonized by parasitic fungi, and some primary saprophytic fungi replace these pathogens and become well established before the leaves are incorporated into the litter layer. As well as this it is probable that some of the fungi colonizing the net-caught leaves were originally present as spores on the live leaves but upon senescence became established as primary colonizers.

After leaf fall, the leaves are rapidly colonized by the litter microflora. At this time there must be considerable competitive interaction between the fungi inhabiting the leaves before leaf fall and the natural microflora and fauna of the forest floor. *Cladosporium* spp., *P. spermatiospora*, and *A. pullulans* are examples of fungi inhabiting leaves before fall which persist after the leaves have come in contact with the forest floor. In fact, *Cladosporium* spp. were shown in this study to increase in frequency of occurrence when the fallen leaves have become part of the L_1 layer. This increase, indicated by isolation data, may either be due to heavy sporulation as members of this genus compete for now less-available nutrients or may be due to the greater ability of *Cladosporium* spp. to compete with the members of the litter mycoflora. However, the competitive abilities of *A. pullulans*, *Cladosporium* spp., and *P. spermatiospora* seem to be affected by the soil microfungi during the winter months (i.e., several months after incorporation into the L_1 layer). *Aureobasidium pullulans* and *P. spermatiospora* were rarely isolated from the litter layer in the spring, while the frequency of occurrence of *Cladosporium* spp. decreased from 30% in the L_1 layer in the fall to 10% in the L layer in the spring. This decrease in frequency of occurrence from the fall to the spring may be a result of the sensitivity of these organisms to the winter conditions and changing physical and biological factors (e.g., pH, substrate), or it may be due to competition from the natural litter microflora. In this connection, it is interesting to note that Hogg and Hudson (1966) also observed that *Cladosporium* spp. persisted on beech litter until the spring after leaf fall. *Guignardia fagi* and *Mycosphaerella tassiana*, the perfect states of *Aureobasidium pullulans* and *Cladosporium herbarum*, were isolated from beech litter by Hogg and Hudson (1966) but were not isolated from the aspen poplar litter in the spring. The absence of the perfect states of these ascomycetes in this present study was possibly due to a difference in isolation technique used, the damp-chamber technique of Hogg and Hudson (1966) favoring the appearance of ascocarps.

The litter fungi that colonized the fallen leaves (L_1) in October included *Beauveria bassiana*, *Phoma* spp., *Discula* spp., *Mortierella* spp., *Phialophora* spp., sterile dark spp., and some *Penicillium* spp. *Beauveria bassiana*, *Phoma* spp., and the sterile dark spp. persisted into spring, while *Discula* spp., *Mortierella* spp., *Phialophora* spp., and the *Penicillium* spp. appeared to disappear from the L_1 during the winter. Species of fungi that appeared on the litter in the spring

but were not isolated from the L_1 with a high frequency of occurrence in the fall included *Paecilomyces* spp. and *Phialophora fastigiata*. Hering (1965) also reported such soil fungi as *Phoma*, *Paecilomyces*, and *Penicillium* spp. from the four types of leaf litter he studied.

Many of the species of the mycoflora initially appearing in the L_1 layer are probably primary saprophytes utilizing those readily available compounds (sugars, etc.) that were not used by the parasitic fungi and other initial colonizers. It is possible that as the L_1 leaves decompose during the winter, these species are replaced by fungal species that have the ability to degrade cellulose.

Fungi isolated from the L_1 layer, such as *Phoma* spp., *Discula* spp., *Mortierella* spp., sterile dark spp., and some *Penicillium* spp., persisted into the L_2 layer, a layer which probably represents the L layer of the previous spring. The frequency of occurrence of *Mortierella* spp. decreased considerably from the L_1 to the L_2 layer, but this decrease was only slight for the other four groups of fungal species. In addition to the fungi that persisted from the L_1 to the L_2 layer, other organisms were also isolated from the L_2 layer material; these included *Alternaria* spp., QL2, *Sclerotium* spp., *Trichoderma* spp., *Mucor* spp., *Penicillium syriacum*, and *Paecilomyces farinosus*. *Penicillium syriacum* was the dominant fungus in the L_2 layer.

It is highly likely that the fungi isolated from the L_2 layer are cellulose decomposers and associated secondary saprophytes. Probably the situation in the L_2 layer can be compared with stage 2 in the decomposition of the leaf substrate in Garrett's (1963) scheme. *Alternaria tenuis* and *Trichoderma viride* have been shown to be strongly cellulolytic (White *et al.* 1948). Many of the dark-pigmented fungi such as *Phoma* spp., QL2, and some sterile dark fungi may also break down cellulose. Secondary saprophytes utilizing the products of cellulose breakdown may include such fungi as *Mortierella* spp. and *Mucor* spp.

Most of the fungal species in the L_2 layer persisted into the F_1 layer. The frequency of occurrence of *Phoma* spp., *Trichoderma* spp., and *P. syriacum* increased and that of *Discula* spp. and *Paecilomyces* spp. decreased from the L_2 to the F_1 layer.

The role of fungi in the F_1 layer is probably that of cellulose, and possibly of lignin, decomposition. *Mucor* spp., *Absidia* spp., and *P. syriacum* probably represent another group of secondary saprophytes associated with the cellulose and lignin decomposers and utilizing the products of such decomposers.

Cellulose and lignin decomposition, with associated secondary saprophytic action, are probably the main functional features of the F_2 and H layers in the aspen poplar forest. As in the F_1 layer, the mycoflora of the F_2 layer was characterized by *Phoma* spp., *Trichoderma* spp., *Mucor* spp., *Absidia* spp., *P. syriacum*, and *Paecilomyces* spp. In addition to these fungi characteristic of the F_2 layer, *Acremonium* spp. and *Volutella ciliata* were also found to be significant components of the mycoflora. *Phoma* spp., *Trichoderma* spp., and *Absidia* spp. were isolated more frequently in the F_2 than in the F_1 layer, while the frequency of occurrence of *P. syriacum* decreased. The frequency of occurrence for many of the dominant fungi from the F_2 layer was about the same at both sampling times, which indicated that the F_2 layer is qualitatively fairly stable. Exceptions included *Mucor* spp., which increased from fall to spring, and *P. syriacum* and *Acremonium* spp., which decreased. Saito (1956) also recorded species of *Absidia*, *Mucor*, and *Trichoderma* from the F_1 and F_2 layers of a beech forest, while Hering (1965) isolated *Penicillium* spp., *Trichoderma viride*, and *Mucor* spp. from leaf litter that had decomposed for over a year. The fungi that Hering (1965) isolated from 12- to 24-month-old litter indicate that the oak, ash, hazel, and birch leaves had decomposed to a state comparable with that found in the F_1 and F_2 layers of the aspen site under study. It should be mentioned that although the F_1 and F_2 layers of the aspen poplar woodland were quite different morphologically, they were found to be extremely similar with regard to fungal species content.

The final stage of fungal succession on aspen leaves in the LFH horizon occurs in the H layer. *Phoma* spp., *Trichoderma* spp., *Paecilomyces* spp., and *V. ciliata* persisted from the F_2 into the H layer in both seasons, while *P. syriacum* had almost disappeared from the H layer. However, the niche previously occupied by *P. syriacum* may have been replaced by other *Penicillium* spp. in the humus. Although *Mucor* spp. were rarely isolated from the humus in both the fall and spring, another phycomycete, *Mortierella*, occurred with a frequency of about 25%. *Cylindrocarpon* spp. also appeared in the humus layer

and occurred with about the same frequency in both the spring and fall samples.

The humus probably compares with stages 2 and 3 of Garrett's (1963) successional scheme. It is quite likely that both cellulose and lignin are undergoing active decomposition by humus layer fungi, as is chitin from the exoskeletons of dead microfauna and dead fungal hyphae. These cellulose, lignin, and chitin decomposers probably have associated with them secondary "sugar fungi" (Hudson 1975). The F_2 and H layers of other types of litter (e.g., coniferous litter, Kendrick and Burges 1962; Parkinson and Balasooriya 1967; Widden and Parkinson 1973) have been reported to follow the same successional pattern as that found in the F_2 and H layers of the aspen poplar litter.

According to the scheme presented by Hudson (1968), the data on successional patterns of fungi on aspen poplar leaves can be summarized in the following way:

Living aspen leaves	Senescent aspen leaves	Dead aspen leaves
Parasites		
Ascomycetes and fungi imperfecti, may be host-specific or host-restricted	Common primary saprophytes	
Sterile dark LL1	Ascomycetes and fungi imperfecti	
Pleurophomella spermatiospora	*Penicillium janthinellum*	Secondary saprophytes
	Cladosporium spp.	Ascomycetes and fungi imperfecti
	Aureobasidium pullulans	*Penicillium syriacum*
	Beauveria bassiana	*Trichoderma* spp.
	Alternaria spp.	*Phoma* spp.
	Sterile dark forms	*Mortierella* spp.
		Paecilomyces spp.
		Mucor spp.
		Volutella ciliata
		Cylindrocarpon spp.

It should be noted that basidiomycetes were very rarely isolated in this study. However, their mycelia were often observed on agar films from the L_2, F_1, and F_2 layers (up to 30% of the total observed mycelia in some cases). This frequent observation of basidiomycete mycelia is probably indicative of their importance in the breakdown of cellulose and lignin in the litter layers of the aspen woodland.

Quantitative Data

The quantitative results obtained at each of the two sampling times are summarized in Table 3. Figures have been expressed as metres mycelium per gram dry weight of organic matter, milligrams wet mycelium per gram dry organic matter, and gram wet mycelium per square metre. Mycelial lengths measured in June displayed a statistically significant ($P = 0.05$) difference between all the layers under study except the F_1 and F_2 layers, while mycelial lengths in the fall sample showed a statistically significant ($P = 0.05$) difference between all layers except the L_2, F_1, and F_2 layers. Mycelial lengths did not significantly vary spatially among the five replicates in either of the two seasons studied.

The quantitative data indicate that the mycelial lengths, and therefore fungal biomass, per gram dry weight of litter were generally higher in the fall than in the spring. The only layer in which the amount of mycelium remained relatively constant in both seasons was the humus (H) layer. This may indicate that the humus is quite stable microbiologically, a feature also indicated by the qualitative data discussed previously. The other layers may be more subject to varying physical, chemical, and biological factors in the soil such as temperature and moisture extremes, pH, and substrate changes.

It is interesting to note that the mycelial content in the layers of the LFH horizon measured in the June samples increased with depth in the horizon from the live leaves to the humus layer. This phenomenon was also observed by Minderman and Daniels (1967) and was explained by the increased average "life-span" of mycelium with depth (Nagel-de Boois 1967). An increase in fungal content from the L_1 layer to the H

TABLE 3

Quantitative data from the aspen poplar LFH horizon

Layer	Litter moisture, % wet wt.	Mycelial lengths, m/g dry litter	Average width of hyphae, μ	Mycelial biomass,* mg/g dry litter	Dry wt. of litter, g/m²	Mycelial biomass, g wet wt./m²
			June			
Live leaves	71.1	13	2.66	0.08		
Litter	11.9	589	2.89	4.34	210	0.91
Fermentation 1	61.5	3062	3.40	31.20	469	14.63
Fermentation 2	66.1	3788	3.24	35.00	601	21.03
Humus	67.1	6249	3.01	49.90	3932	196.21
			October			
Net-caught leaves	70.3	418	2.66	2.61		
Litter 1	79.2	4773	2.89	35.10	215†	7.55
Litter 2	67.3	7935	3.40	80.90	210	16.99
Fermentation 1	71.8	7930	3.40	80.80	469	37.90
Fermentation 2	69.4	7215	3.24	66.80	601	40.15
Humus	66.2	5996	3.01	47.90	3932	188.34

*The specific gravity used to calculate the mycelial biomass was the mean of three specific gravity figures obtained by Saito (1955) from three different species of fungi.
†Litter input (Parkinson and Lousier 1974).

layer was, however, not observed in October. Instead, mycelial lengths were much higher in the L_2, F_1, and F_2 layers than in the H layer. The high mycelial lengths measured in the litter and fermentation layers at the October sampling time may have been partially due to an increase in nutrient status contributed by the freshly fallen leaves. However, these high mycelial measurements may have resulted from a rise in moisture content caused by snowfall just before sampling. The data obtained cannot be further explained unless a complete study is made of the effects of various physical, chemical, and biological factors on the growth rates and decomposition rates of fungal mycelium.

However, the quantitative data obtained in both June and October can be used to approximate the minimum change in standing crop of mycelium from one season to the next, or in one case can be used to estimate the minimum change in standing crop of mycelium from one layer to the next. For example, there was a considerable increase in standing crop of mycelium from the time the leaves fell from the trees to the time that they were incorporated into the L_1 layer. From the data presented in Table 3, it can be seen that there was a difference of 4355 m of mycelium or 32.49 mg fresh weight mycelium per gram dry litter between the net-caught leaves and the L_1 leaves. The net-caught and L_1 leaves were essentially the same litter except that the L_1 leaves had been exposed to the litter microflora for 2 to 3 weeks after leaf fall, whereas the netted leaves were not. Thus these data indicate the rate of initial colonization of leaf litter by fungi, a rate which is presumably much affected by prevailing moisture and temperature conditions at the litter surface. (The moisture conditions at the time of this study are given in Table 3.) There also seems to be an estimated increase of 405 m or 2.53 mg mycelium per gram dry weight leaf material from the time the leaves came out of bud (live leaf sample) to just before their reaching the soil surface (net-caught leaves).

If it is assumed that the dry weight of 1 m^2 of organic matter in the L_2, F_1, F_2, and H layers remained relatively constant from June until October, it is possible to estimate in very crude terms the change in standing crop of mycelium in grams per square metre dry litter during this 5-month period. Assuming the L_2 layer sampled in October was actually the L layer in June, the amount of mycelium produced over the summer in this litter layer was about 16 g/m^2 dry litter. The largest increase in mycelial content occurred in the F_1 layer where about 23 g of mycelium per square metre on a dry weight basis was produced from spring to fall. The increase in standing crop of mycelium from June until October in the F_2 layer amounted to about 19 g/m^2. The humus was the only layer in which a decrease in standing crop of mycelium (0.8

g/m^2) was recorded in the period from spring to fall. The data provided indicate that total increase in standing crop of mycelium (June to October) in the LFH horizon is at least 57 g wet weight mycelium per square metre dry litter.

The large increases in mycelial biomass in the L_2, F_1, and F_2 layers from June until October indicate the importance of the fungi in the decomposition of aspen litter. A large amount of energy appears to be transformed from the organic matter into mycelial biomass during the summer and early fall months, thereby indicating the significance of fungi in the energy transfer from one trophic level to the next in the aspen woodland ecosystem. As well as this, significant concentration of nutrients will occur in the fungal biomass. However, it must be emphasized that the estimates given here on change in fungal standing crop are at best very crude. Any refinements of these and subsequent estimates on energy transfer and nutrient immobilization require accurate data on respiration, decomposition, and growth rates of the fungi under field conditions.

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